http://dx.doi.org/10.4314/jpb.v9i1.7
Vol. 9 no. 1, pp. 34-38 (March 2012)
http://ajol.info/index.php/jpb

Journal of
PHARMACY AND BIORESOURCES

Analgesic and anti-inflammatory activity of the aqueous leaf extract of *Piliostigma thonningii* (Caesalpinoideae)

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Received 13th January 2012; Accepted 9th March 2012

Abstract
The leaves of *Piliostigma thonningii* (Schum) have been used in herbal medicine to arrest bleeding, as laxatives, to treat fevers, bacterial infection and inflammation. In order to provide scientific justification for the ethnomedical uses of the leaves, the study was designed to investigate the analgesic and anti-inflammatory activity of the extract and its possible mechanism of action. Analgesic activity was examined using the tail immersion and acetic acid-induced writhing test while acute anti-inflammatory effect was studied using xylene-induced ear edema model. The extract significantly ($p<0.05$) decreased the number of writhes in the mice at 200 and 400 mg/kg doses of the extract with 54.95 and 56.53% inhibition respectively but did not show any significant effect ($p>0.05$) in the tail immersion test when compared to the control. There was a significant decrease ($p<0.001$) in ear oedema induced by xylene by 200 and 400 mg/kg of the extract with 83.06 and 90.55% inhibition respectively. Oral acute toxicity assays did not show any mortality at 10 g/kg of the plant extract. The inhibition of acetic acid-induced writhing in mice by extract suggested that its analgesic affect maybe be peripherally mediated and the inhibition of edema suggests a likely indication of the antiphlogistic effects of the extract. In conclusion, the leaf extract of *P. thonningii* possesses anti-inflammatory and analgesic properties which may be mediated via peripheral inhibiting mechanisms. These results thus justify its use in the treatment of pain and inflammatory conditions in tradomedical practice.

Keywords: *Piliostigma thonningii;* anti-inflammatory; analgesic.

INTRODUCTION

Several herbal medicines obtained from various plant extracts are being used in the treatment of a wide variety of clinical diseases, though relatively little knowledge is known absent their mechanism of action (Ratheesh & Helen, 2007). Many herbal preparations are also being prescribed widely for inflammatory conditions (Bagul *et al*., 2005).

Piliostigma thonningii Schum (family Caesalpinoideae; common names, monkey bread and camel's feet) is a small tree up to 10m high with leaves that are simple, apically bilobed with rust-coloured indumentum breath, 7.5–15cm long and 8–10m broad. In Nigeria, *P. thonningii* is known as Okpoatu in Ibo, Kalgo in Hausa and Abefe in Yoruba (Togola *et al*., 2005). In Nigeria, the leaves and stem bark are being used to treat cough toothache, fever, rheumatism, ulcers, as an anthelmintic and as a diuretic (Togola *et al*., 2005; Igoli *et al*., 2005; Gill, 1992). The aqueous leaf extract has been reported to

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ISSN 0189-8442

increase locomotor activity, reduce bleeding time and constrict aortic rings (Ozolua *et al*., 2009). D-3-*O*-methyl chiroinositol isolated from the stem bark is active against *Haemoncus contortus* (Asuzu *et al*., 1999). C-methylflavenols isolated from the leaves have been reported to have both antibacterial and anti-inflammatory activity (Ibewuike *et al*., 1997).

The seed extracts have been shown to be active against the influenza virus and herpes simplex virus type I (Silva *et al*., 1999). Although the aqueous leaf extract has been used traditionally in treatment of headache and back ache, there is no scientific evidence establishing the mechanism of action of the crude aqueous extract. Hence the present study was undertaken to evaluate the analgesic and anti-inflammatory activity of the leaf extract and its possible mechanism(s) of action.

EXPERIMENTAL

Plant material and extraction. The leaves of *P. thonningii* were collected from Owan village in Ovia East Local Government area in Edo State in the month of June and were identified and authenticated by Dr. E. J. Aigbokhan of the Department of Plant Biology and Biotechnology, Faculty of Life Sciences, University of Benin where herbarium specimen has been deposited. The leaves were dried on the laboratory bench for 28 days and then powdered using a mechanical grinder. The powdered leaves (538.4g) were extracted by hot maceration at 80^{o}C for 30 min in 2.5L of distilled water, with stirring at regular intervals. The filtrate was concentrated in a rotary evaporator at 70^{o}C and then dried in an oven set at 40^{o}C. The crude extract obtained was stored in the refrigerator at 4^{o}C until needed.

Animals. Experiments were performed using Swiss albino mice (16-26g) of either sex. The animals were obtained from the Laboratory Animal Centre, University of Ibadan, Oyo State, Nigeria. The animals were fed with standard rodent cubes obtained from Ladokun Feeds Ltd. Ibadan, Nigeria and had free access to tap water. All animals were fasted over night before the beginning of each experiment. Animals were exposed to natural light conditions and were handled according to standard experimental protocols approved by the Faculty of Pharmacy Animal Ethics Committee, University of Benin, Nigeria.

Drugs and chemicals. Acetylsalicylic acid, acetic acid (96%) (Sigma Aldrich, Germany,) dexamethasone (Embassy, Nigeria), Chloroform (Riedel-de Hean, Germany), distilled water, xylene (BDH chemicals, Poole England), and acacia gum powder (Harlewood chemical, England) were used as obtained.

Acute toxicity tests. Swiss albino mice (16-22 g) of either sex, fasted over night were used for the study. The animals were divided into four groups of five animals each. Group A to C received orally 1, 5, and 10 g/kg of extract respectively, while the control (group D) received distilled water (3 ml/kg) by same route. General symptoms of toxicity and mortality in each group were observed within 24 h. Animals that survived after 24 h were observed for any signs of delayed toxicity for two weeks (Miller and Tainter, 1944).

Analgesic activity

Mouse writhing test. The analgesic effect of extract was evaluated by the acetic acid-induced mouse writhing test (Koster *et al*., 1999). The extract (200 or 400 mg/kg), acetyl salicylic acid (100 mg/kg) or distilled matter (10 ml/kg) were administered orally to the animals 1 h, before intraperitoneal injection of acetic acid (0.6% v/v). The number of writhes by each mouse was counted immediately after acetic acid administration at intervals of 5 min for a period of 30 min.

Tail immersion test. In the tail immersion test (Janssen *et al*., 1963) the tail (up to 5cm) of each mouse was immersed in water bath

thermostatically maintained at 55^{o}C. The withdrawal time of the tail from hot water was noted as the reaction time or tail flick latency. The plant extract (200 or 400 mg/kg), distilled water (10 ml/kg) and morphine (10 mg/kg) were administered intraperitoneally to four groups of mice respectively. The reaction time was recorded before and 30, 60 120 and 180 min after administration and then compared with that of the control.

Anti-inflammatory activity

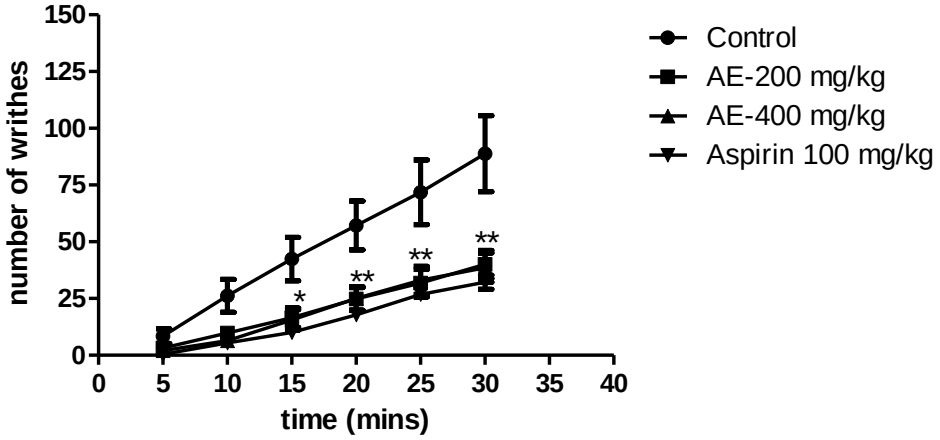
Xylene-induced ear oedema. Swiss albino mice were divided into four groups of five animals each. Animals were treated orally with the extract (200 or 400 mg/kg), dexamethasone (1 mg/kg) and distilled water (3 ml/kg). One hour later, oedema was induced in each mouse by applying a drop of xylene on the inner surface of the right ear. After 15min, the animals where sacrificed and both ears were cut off and weighed (Igbe *et al*., 2010). The anti-inflammatory activity was expressed as the percentage inhibition of oedema in the treated groups to that of the control group.

Statistical analysis. Data were expressed as the mean $\pm$ SEM. The data were analyzed using one way analysis of variance (ANOVA) followed by Turkey's test. Differences between two means were detected using the student's t-test. Data were considered different at significance level of $p>0.05$.

RESULTS

Acute toxicity study showed that all doses (1, 5 and 10 g/kg) of the *P. thonningii* extract used for the study was non toxic. In the acetic acid induced writhing test (Fig. 1), the extract significantly ($p<0.05$) decreased the number of writhes in the mice at 200 and 400 mg/kg doses of the extract with 54.95 and 56.53% inhibition respectively. The extract (200 and 400 mg/kg) did not show any significant effect ($p>0.05$) in the tail immersion test (Table 1) when compared to the control but there was a significant decrease ($p<0.001$) in ear oedema induced by xylene (Table 2) by 200 and 400 mg/kg of the extract with 83.06 and 90.55% inhibition respectively when compared to the control.

Fig 1. Effect of aqueous extract of *Piliostigma thonningii* on acetic acid-induced mouse writhing.



*p < 0.05, **p<0.01 as compared to the corresponding time point for control. n = 5.

Table 1. Effect of aqueous extract of *P. thonningii* on pain threshold of mice in tail immersion test

Treatment	Dose (mg/kg)	Before treatment	Time after treatment (min)			
			30	60	120	180

Control	10 mL/kg	2.59 ± 0.43	2.26 ± 0.22	2.31 ± 0.55	2.04 ± 0.63	1.91 ± 0.40
P. thonningii	200	2.24 ± 0.09	2.33 ± 0.28	1.96 ± 0.27	1.94 ± 0.38	1.63 ± 0.32
P. thonningii	400	1.82 ± 0.24	2.31 ± 0.26	2.54 ± 0.67	4.39 ± 0.98	3.20 ± 0.93
Morphine	10	1.92 ± 0.40	10.09 ± 0.77***	13.64 ± 0.80***	11.75 ± 0.38***	9.24 ± 0.68***

Data are the mean ± SEM values for five mice in each group. *** $p < 0.01$ as compared to the control

Table 2. Effect of aqueous extract of *P. thonningii* on xylene-induced ear edema in mice

Treatment	Dose (mg/kg)	Weight of right ear (mg)	Weight of left ear (mg)	Difference (mg)	Inhibition (%)
Control	10 mL/kg	58.24 ± 4.19	29.02 ± 2.45	29.22 ± 4.15	-
P. thonningii	200	30.34 ± 1.42	27.26 ± 2.90	4.95 ± 1.87***	83.06
P. thonningii	400	32.08 ± 1.87	31.82 ± 2.84	2.76 ± 0.71***	90.55
Dexamethasone	1	33.84 ± 1.93	28.54 ± 0.20	4.40 ± 1.74***	84.94

Data are the mean ± SEM values for five mice in each group. *** $p < 0.01$ as compared to the control

DISCUSSION

In the present study, the analgesic and anti inflammatory activity of the aqueous leaf extract of *P. thonningii* have been evaluated using various animal models. The acetic acid-induced mouse writhing test has been used extensively to qualify analgesic agents that have peripheral analgesic activity (Neves *et al*., 2007). Writhing induced by chemical substances injected intraperitoneally, are due to sensitization of nociceptors by prostaglandins. Inhibition of acetic acid-induced writhing in mice by extract (200 and 400 mg/kg) suggested that the analgesic affect of the extract maybe be peripherally mediated via the inhibition of the synthesis and release of prostaglandins (Koster *et al*., 1999). The extract (200 and 400 mg/kg) failed to increase mice reaction time in the tail immersion test compared to morphine (10 mg/kg) which showed significant antinonciceptive activity 30 min after treatment. It is known that centrally acting analgesic drugs elevate the pain threshold of mice toward heat and pressure[14]. Since the extract did not raise the pain threshold in the tail immersion test, this indicated that it may not be acting centrally. Hence the extract seems to possess analgesic properties, which are mediated via peripheral inhibitory mechanisms. The xylene ear oedema model permits the evaluation of anti-inflammatory steroids and is less sensitive to non-steroidal anti-inflammatory agents (Adeyemi *et al*., 2004). Histopathologically, severe vasodilation, edematous changes of skin and infiltration of inflammatory cells are detected as signs of acute inflammatory response after topical application of xylene (Zaninir *et al*., 1992). In the present study, the increases in ear weight were inhibited by the extract (200 and 400 mg/kg) in a dose related manner, thus suggesting a likely indication of the antiphlogistic effects of the extract. The effect of the extract in this model suggests inhibition of phospholipase A_2, similar to that provided by anti-inflammatory steroids such as dexamethasone.

The presence of the reported phytochemical constituents in the fruit pulp extract may contribute to its observed anti-inflammatory activity. This is based on the fact that many flavonoids and alkaloids have been found to exhibit anti-inflammatory effects (Martini *et al*., 2004; Igbe *et al*., 2010; Kou *et al*., 2005). The C-methylflavonols which have been found as constituents of the leaves of *Piliostigma thonningii* have been reported to inhibit the synthesis of prostaglandins and hence this may be responsible for its anti-inflammatory activity (Ibewuike *et al*., 1997).

In conclusion, it has been established that the aqueous leaf extract of *Piliostigma thonningii* possessed analgesic properties which are mediated via peripheral inhibiting mechanisms. The ability of the extract to inhibit inflammatory responses produced in the xylene-induced ear oedema model shows

that it possessed anti-inflammatory properties. These results thus justify its use in the treatment of pain and inflammatory conditions such as tooth ache and back ache in tradomedical practice.

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